

RESEARCH NOTE

VIROLOGY

Human papillomavirus (HPV) infection and genotype frequency in the oral mucosa of newborns in Milan, Italy

M. Martinelli¹, A. Zappa¹, S. Bianchi¹, E. Frati¹, D. Colzani¹, A. Amendola^{1,2} and E. Tanzi^{1,2}

1) Department of Public Health-Microbiology-Virology, University of Milan, Milan and 2) CIRI-IV, Department of Health Sciences, University of Genoa, Genoa, Italy

Abstract

Human papillomavirus (HPV) causes cutaneous and mucosal infections in both adults and children. In order to evaluate HPV prevalence and the spectrum of genotypes in the oral cavity of paediatric subjects, a retrospective study was carried out on oral-pharyngeal swabs collected from 177 newborns aged 0–6 months. HPV-DNA was detected by a nested-PCR; the viral typing was made through DNA sequencing. HPV infection was identified in 25 subjects (14.1%) and the sequence analysis showed eight distinct genotypes. These data confirm HPV detection in newborn oral mucosa. Further investigations are needed to clarify the methods of HPV acquisition.

Keywords: Cutaneous HPV, human papillomavirus, mucosal HPV, newborn infections, oral HPV

Original Submission: 22 December 2011; **Revised Submission:** 1 March 2012; **Accepted:** 7 March 2012

Editor: L. Kaiser

Article published online: 12 March 2012

Clin Microbiol Infect 2012; **18**: E197–E199

10.1111/j.1469-0691.2012.03839.x

Corresponding author: E. Tanzi, Dipartimento di Sanità Pubblica-Microbiologia-Virologia, Università degli Studi di Milano, Via Pascal 36, 20133 Milan, Italy
E-mail: elisabetta.tanzi@unimi.it

Human papillomaviruses (HPVs) are small double-stranded DNA viruses, grouped into cutaneous and mucosal types according to the infection site, with a further subdivision into high-risk (HR) and low-risk (LR) genotypes, depending

on their association with malignancy [1]. HPV is a common pathogen in adults, but it is also associated with a variety of mucosal and skin infections in the paediatric population. Several studies have confirmed the detection of HPV in children, though the rates reported are controversial. HPV DNA has been found in oral swabs of newborns in percentages varying from 4% to 87% [2,3]. HPV infection in infant oral mucosa seems to be acquired at birth [2], probably due to exposure to the mother's cervical HPV infection. The oral infection persists for at least 6 months, with a decreasing rate during the first 3 years of life [1,3–5]. Other potential ways of transmission, such as auto- and hetero-inoculation, sexual abuse [6,7] and indirect transmission via fomites [7], are only hypothetical for the paediatric population.

This study aims to assess the prevalence of HPV infection and viral genotypes affecting the oral cavity of paediatric subjects.

A retrospective study was carried out on oral-pharyngeal swabs collected during a previous epidemiological-molecular surveillance of respiratory infections in children. Samples were collected from hospitalized children and routinely tested for diagnosis of respiratory illness from 2009 through to 2010 [8]. Written informed consent was obtained from parents or legal guardians for each child included in the study so as to store their samples for further anonymous research testing.

Human papillomavirus testing was retrospectively performed in 2011 on a subgroup of 177 samples belonging to the population of babies aged 0–6 months. Among these newborns, 58.8% (104/177) were male and 41.2% (73/177) female. The median age was 3 months (IQR, 1–4 months). In order to evaluate the age distribution of HPV infection, the children were divided into seven age groups: 0–1 month (14.7%; 95% CI, 10–20.5%), 1–2 months (18.1%; 95% CI, 12.9–24.3%), 2–3 months (16.4%; 95% CI, 11.5–22.4%), 3–4 months (23.7%; 95% CI, 17.9–30.4%), 4–5 months (11.3%; 95% CI, 7.2–16.6%), 5–6 months (7.9%; 95% CI, 4.6–12.6%) and 6–7 months (7.9%; 95% CI, 4.6–12.6%).

DNA was extracted from oral-pharyngeal samples by using a commercial kit (NucliSENS[®] miniMAG[®], bioMérieux bv, Lyon, France). The concentration and purity of the extracted DNA was evaluated through a spectrophotometer (NanoDrop ND-2000/2000C, Euroclone[®], Thermo scientific, Wilmington, DE, USA). HPV-DNA was detected through a specific nested-PCR assay amplifying a 150 bp segment of ORF L1 using the two primer pair: MY09/MY11 and GP5+/GP6+ [9,10]. Each run was accompanied by positive (HPV-16 positive cells, Caski) and negative (water) control samples. The DNA integrity was assessed by amplification of a 268 bp

fragment in the ubiquitous beta-globulin gene using the primer pair GH₂₀ and PCO₄. The amplification products were visualized using electrophoresis analysis in a 2% agarose gel containing ethidium bromide (0.5 mg/L) and compared with a standard (DNA Molecular Weight, Marker 100, Sigma-Aldrich, St. Louis, MO, USA). The PCR products of approximately 150 bp from the revised LI consensus PCR assays underwent genotyping through automated DNA sequencing on the ABI PRISM 3100 genetic analyser (Applied Biosystems, Carlsbad, CA, USA). Data were expressed as median (interquartile range, IQR) and percentages (95% confidence intervals, 95% CI) where appropriate. Comparisons between groups were performed using the chi-square test or Fisher's exact test. A *p*-value <0.05 was considered statistically significant (two-tailed test). All statistical analyses were performed using OpenEPI software, version 2.3.1 [11].

The overall prevalence of HPV infection among newborns was 14.1% (25/177; 95% CI: 9.6–19.9%). The median age of HPV-positive children was 4 months (IQR, 3–4 months), 52% (13/25; 95% CI, 32.8–70.8%) were female and 48% (12/25; 95% CI, 29.2–67.3%) were male. Table 1 shows HPV-positive rates and the identified genotypes by age group.

Amplified DNA from 14 out of 25 (56%) positive samples underwent DNA sequencing and eight distinct HPV genotypes were detected, five of which belonged to the mucosal HR-HPV types (HPV-16, -18, -31, -33 and -58) and two to the mucosal LR-HPV types (HPV -81 and -84) (Table 1). HPV-81 was the prevalent mucosal genotype, identified in 5/25 (20%; 95% CI, 7.7–38.9%; *p* 0.13) samples (Fig. 1). Two oral-pharyngeal swabs (8%) showed the cutaneous genotype HPV-27.

The prevalence of HPV infection (14.1%) found in this study is included in the wide spectrum of rates reported for oral mucosa of paediatric populations [1,3,12]. This variability depends on the type of the population studied, the type of the sample analysed and the methods used for HPV detection [13]. As for age, we found a higher rate of infection (40%) in subjects aged 4–5 months than in

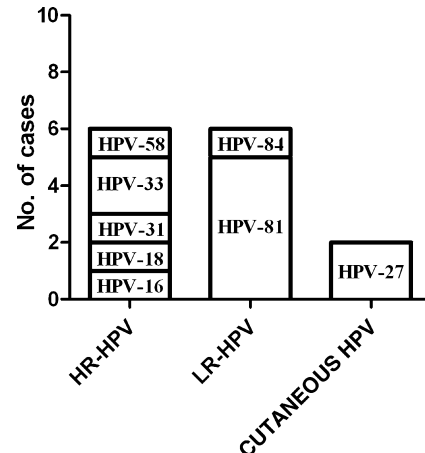


FIG. 1. HPV genotypes identified in newborn oral mucosa.

other age groups (*p* 0.01). The increasing exposure of infants of this age to external contacts, beyond those with their own mother, could explain this finding, but the number of individuals tested is too low to make definitive conclusions.

The same percentage (24%) of genotypes at high (HR) and low (LR) oncogenic risk was associated with the mucosal oral-pharyngeal HPV infections detected. The prevalent genotype was LR-HPV-81, found in 20% of children, particularly those aged 3–4 months. The identification of high-oncogenic-risk HPV genotypes in newborns' respiratory tracts has been reported [14,15] and the genotypes identified match those frequently infecting the genital tract of childbearing-age women [16]. This may support a potential transmission of HPV infection from mothers to children. However, as a limitation of this study, the lack of information concerning the HPV status of the mothers does not allow definitive conclusions to be drawn.

Long-term longitudinal studies could also clarify the natural history of HPV infection in the oral cavity of paediatric subjects. The presence of viral genotypes in this site may be associated with transient infections, similar to those affecting

TABLE 1. Distribution of HPV-positive rates and genotypes by age group

Age group (months)	No. of children	%; 95% CI (out of 177)	No. of HPV+ individuals	% HPV+ per age group; 95% CI	HPV genotype (No. of positive samples)
0–1	26	14.7; 10–20.5	2	7.7; 1.3–23.2	NT(2)
1–2	32	18.1; 12.9–24.3	3	9.4; 2.4–23.4	HPV-27(2), -81(1)
2–3	29	16.4; 11.5–22.4	1	3.4; 0.2–15.9	HPV-84 (1)
3–4	42	23.7; 17.9–30.4	6	14.3; 6–27.4	HPV-18(1), -31(1), -33(1), -81(3)
4–5	20	11.3; 7.2–16.6	8	40.0; 20.6–62.1	HPV-16(1), -81(1), NT(6)
5–6	14	7.9; 4.6–12.6	3	21.4; 5.8–48	HPV-33(1), NT(2)
6–7	14	7.9; 4.6–12.6	2	14.3; 2.5–39.7	HPV-58(1), NT(1)

NT, non-typed.

the genital level, or persistent ones, with an increasing risk of progression to symptomatic clinical forms.

The unusual presence of HPV-27 in the oral mucosa of two newborns requires further investigation, being a cutaneous genotype normally associated with the development of common skin warts [12,17]. A possible source of acquisition could be a direct contact with the mother, given the close mother-child relationship in the postnatal period, or an indirect transmission via fomites.

These preliminary data confirm the HPV circulation in newborns. Further studies involving a larger selection of subjects are needed to clarify several crucial points, such as the methods of HPV acquisition and the prognosis of infection.

Acknowledgements

The authors thank Francesco Roncacci for English revision. This work was supported by the Italian Ministry of University and Research (PRIN-2007, protocol 2007LPAF42_003) and by "Dote Ricerca" FSE 2007-2013, Regione Lombardia.

Transparency Declaration

None of the authors have financial or other conflicting interests with regard to the information described in this manuscript.

References

1. Cason J, Mant CA. High-risk mucosal human papillomavirus infections during infancy & childhood. *J Clin Virol* 2005; 32 (suppl 1): 52–58.
2. Syrjänen S, Puranen M. Human papillomavirus infections in children: the potential role of maternal transmission. *Crit Rev Oral Biol Med* 2000; 11: 259–274.
3. Rintala MA, Grénman SE, Järvenkylä ME, Syrjänen KJ, Syrjänen SM. High-risk types of human papillomavirus (HPV) DNA in oral and genital mucosa of infants during their first 3 years of life: experience from the Finnish HPV Family Study. *Clin Infect Dis* 2005; 41: 1728–1733.
4. Mant C, Kell B, Rice P, Best JM, Bible JM, Cason J. Buccal exposure to human papillomavirus type 16 is a common yet transitory event of childhood. *J Med Virol* 2003; 71: 593–598.
5. Rombaldi RL, Serafini EP, Mandelli J, Zimmermann E, Losquiavo KP. Transplacental transmission of Human Papillomavirus. *Viral J* 2008; 5: 106.
6. Sinclair KA, Woods CR, Kirse DJ, Sinal SH. Anogenital and respiratory tract human papillomavirus infections among children: age, gender, and potential transmission through sexual abuse. *Pediatrics* 2005; 116: 815–825.
7. Syrjänen S. Current concepts on human papillomavirus infections in children. *APMIS* 2010; 118: 494–509.
8. Zuccotti G, Dilillo D, Zappa A et al. Epidemiological and clinical features of respiratory viral infections in hospitalized children during the circulation of influenza virus A(H1N1) 2009. *Influenza Other Respi Viruses* 2011; 5: 528–534.
9. Manos M, Ting Y, Wrigth DK, Lewis AJ, Broker TR, Wolinsky SM. Use polymerase chain reaction for the identification of genital human papillomaviruses. *Cancer Cell* 1989; 7: 209–214.
10. Qu W, Jiang G, Cruz Y et al. PCR detection of human papillomavirus: comparison between MY09/MY11 and GP5+/GP6+ primer systems. *J Clin Microbiol* 1997; 35: 1304–1310.
11. Dean AG, Sullivan KM, Soe MM. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version 2.3.1. <http://www.OpenEpi.com> (last accessed 28 February 2012).
12. Mammas IN, Sourvinos G, Spandidos DA. Human papilloma virus (HPV) infection in children and adolescents. *Eur J Pediatr* 2009; 168: 267–273.
13. Kurose K, Terai M, Soedarsono N et al. Low prevalence of HPV infection and its natural history in normal oral mucosa among volunteers on Miyako Island, Japan. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004; 98: 91–96.
14. Smith EM, Parker MA, Rubenstein LM, Haugen TH, Hamsikova E, Turek LP. Evidence for vertical transmission of HPV from mothers to infants. *Infect Dis Obstet Gynecol* 2010; 2010: 326369.
15. Sinal SH, Woods CR. Human papillomavirus infections of the genital and respiratory tracts in young children. *Semin Pediatr Infect Dis* 2005; 16: 306–316.
16. Giorgi Rossi P, Chini F, Bisanzio S et al. Distribution of high and low risk HPV types by cytological status: a population based study from Italy. *Infect Agent Cancer* 2011; 6: 2.
17. Ostrow RS, Zachow KR, Shaver MK, Faras AJ. Human papillomavirus type 27: detection of a novel human papillomavirus in common warts of a renal transplant recipient. *J Virol* 1989; 63: 4904.